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The Marine Laboratory
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SEMI-ANNUAL PROGRESS REPORT
TO THE OFFICE OF NAVAL RESEARCH

February 1953

Contract NConr-705 (00)
Project NR 165-304

MARINE BORER PROJECT



CORAL GABLES, FLORIDA

THE MARINE LABORATORY

UNIVERSITY OF MIAMI

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Coral Gables, Florida

ML 4694

F. G. Walton Smith

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Director

CONTENTS

	Page
1. Summary	1
2. Capillary Microrespirometry	2
3. Cellulase Enzyme System of Adult <u>Teredo</u>	3
4. Pumping Rate of Adult <u>Teredo</u>	4
5. X-ray Studies	4
6. Accelerated Leaching Tests	5
7. Miscellaneous Panel Exposure Tests	11
8. Publications	13

Chart I

Figures 1 - 9

SEMI-ANNUAL PROGRESS REPORT

February 1953

1. Summary

During the period covered by this report the toxicity of whole creosote has been studied in the capillary microrespirometer using individual Teredo larvae. It has been shown that a linear relationship exists between log concentration and oxygen uptake between the limits 5×10^{-7} gm/ml and 5×10^{-12} . The minimal effective dose of creosote, under the conditions of these experiments lies between 5×10^{-11} and 5×10^{-12} gm/ml. The significance of these results is discussed.

Evidence is presented which suggests that the total cellulose-digesting ability of Teredo is the consequence of the combined activity of two qualitatively different enzyme systems. One of these is located in the post-cecal portion of the gut and shows a definite pH optimum between pH 5.6 - 6.7. The other appears to be located in the prececal portion of the animal, shows no definite pH optimum and, under the conditions of our experiments, possesses only half the activity of the post-cecal material.

X-ray studies of over two hundred individual living Teredo have provided X-ray characteristics by which more than a single species of borer may be identified. This multiplicity of species has been borne out by taxonomic work. This investigation is continuing.

A summary is given of a considerable amount of data now accumulated with regard to the leaching test. A tentative protocol arising from this was used to evaluate various creosote extracts and fraction fortified whole creosote. The erratic nature of the numerical results casts doubt on the usefulness of this test. No conclusions are drawn, however, prior to com-

pletion of tests still being carried out.

Holiday panels show evidence that creosote gives very slight protection only to adjacent untreated wood within a distance of 1/8".

Panel tests show that Erdalith treatment maintained a high resistance in panels during the test period of two years. Greenheart panels are in generally good condition after 2½ years, although some large empty burrows are present. Some silica containing Surinam timbers show good initial resistance.

A composite panel exposed for 2½ years shows no signs of the penetration of creosote wood by worms in contiguous untreated wood.

2. Capillary Microrespirometry

During the period covered by this report one capillary microrespirometer has been used chiefly to study the toxicity of whole creosote. The material for study was prepared as outlined in the preceding semi-annual report, i.e., homogenization with sea water and then serial dilution of this homogenate with fresh sea water. Individual larvae of 12 hours age are placed in 10 microliters of this medium. Their rate of oxygen uptake is followed during a period of ninety minutes, readings are made at five minute intervals.

Table I includes average values derived from a study of 10 animals at each concentration level. Normal figures are averages of thirty normal animals in sea water, so this table presents average results of a study of 100 Teredo larvae.

Table I

Effects of Creosote on Respiration
of Teredo Larvae

Concentration of Creosote	Average Oxygen Uptake/hr./larva
5×10^{-7} gm/ml	17.13 mm ³
5×10^{-8}	21.15
5×10^{-9}	28.70
5×10^{-10}	34.75
5×10^{-11}	41.70
5×10^{-12}	45.40
0.0 (normal sea water)	47.8

See Chart I . . .

These results provide a pharmacologic standard of reference against which to compare the toxicity, not only of creosote fractions but also of any other toxic material which may be prepared in the future. Future efforts will be concerned with an extensive study of creosote fractions in an attempt to identify components in crude creosote which are responsible for the extreme toxicity which has been demonstrated.

Our figures show that a concentration of creosote between 5×10^{-11} and 5×10^{-12} gm/ml of sea water is the minimal level at which a response can be detected under the conditions of these experiments. Concentrations of the order of 5×10^{-7} gm/ml sea water are uniformly lethal in eight hours.

3. Cellulase enzyme system of Adult Teredo

Further work on this aspect of the general problem has been confined to efforts to define some of the conditions of the activity of the enzyme

system. The pH optimum which was reported in our previous semi-annual report has been confirmed. Additional work has made it quite clear that the total cellulose-digesting ability of Teredo is the consequence of the combined action of at least two enzyme systems. One of these is associated with the pre-cecal portions of the gut. The enzyme produced by this region differs from other cellulase enzymes of Teredo in that no definite pH optimum is shown -- its activity is relatively constant over the range pH 5.0 to pH 7.8. The enzyme complex associated with the post-cecal portion of the gut, on the other hand, shows the typical pH optimum that has been already reported. One further observation of interest is that the enzyme appears to be dependent on adenosine triphosphate for its activity. Further work on this aspect of the problem is under way at present and will be reported at a later date.

4. Pumping Rate of Adult Teredo

Work on this aspect of the general problem has been completed and the results are being prepared for publication as a technical report.

5. X-ray Studies

These observations have now been pursued for one full calendar year. The data are being actively studied at the present time but no specific conclusions can be reached until the statistical portion of the study shall have been completed. It may be mentioned in passing that increasing familiarity with the x-ray images of local shipworms led increasingly to the suspicion that more than a single species of Teredid is native to the Miami area. Taxonomic studies by Mr. Gilbert Voss and others have fully confirmed this impression. Specimens are presently being collected for study both locally and at the Museum of Comparative Zoology at Harvard.

It is anticipated that these studies will make it possible for us to state more accurately than has so far been possible the range of species which are to be found as natives in local waters.

A tentative conclusion justified by repeated observation of the phenomenon in the course of x-ray studies of growth throughout the life span of individual borers, is that the end of life is apparently signaled by spawning. The average length of life in these waters is not far from twelve weeks. During this time the borers function first as ♂ and finally they spawn but once as ♀ and then die.

6. Accelerated Leaching Tests

A new apparatus was constructed with a capacity for simultaneous accelerated leaching at 80°C of approximately two hundred 5" x 1½" x 1/8" panels. The 25 gallon stainless steel tank is equipped with thermoregulators near to each end for control of the temperature of continuously flowing fresh water.

A. Preliminary experiments. With the completion of field tests, data from the first accelerated leaching test were analyzed so that an experimental procedure for subsequent tests could be designed which would yield accurate, reproducible and dependable results. Panels were sent to NRL for determination of residual creosote retention. The procedure for this experiment was outlined in the semi-annual report of February 1952. Creosote treated panels were leached in fresh water for periods of 0, 2, 4, 8, 16, 34 and 48 days and subsequently exposed in the sea to borer attack. They were inspected for borer attack after 1, 2, 3, 4, 6 and 8 months. Figure 1 illustrates the extent of attack on the panels after six months of exposure. Attack is rated from 0 to 5 separately for Limnoria and Teredo. Because of

the greater resistance of panels to Teredo attack, the numerical index for attack rating was arbitrarily reached by adding twice the amount of Teredo attack to the amount of Limnoria attack.

Resistance to borer attack by panels which had been leached for 16 days was a positive function of the creosote concentration in the panels. Panels leached for periods of time less than 16 days, however, did not show such consistent correlations. On the basis of this result, a leaching period of 16 days was chosen as most suitable for the appraisal of the effectiveness of creosote treatments.

Figure 2 illustrates the extent of attack occurring during 8 months of exposure to a series of panels which had approximately equal concentrations of creosote, but which had been leached for different periods of time. Prior to four months of exposure the extent of attack varies independently of the degree of leaching. Attack after 4 months exposure shows a rapid but erratic increase with amount of leaching up to 16 days. After 16 days leaching, attack rate remains fairly constant, with only a slight positive correlation with leaching period. A period of exposure of six months was chosen as the standard for subsequent tests, to allow for a seasonal variation in attack rate.

The data analyses summarized above led to the following tentative basis protocol for evaluating creosote constituents in accelerated leaching tests.

1. Panels (5" x 1½" x 1/8") covering an appropriate range of toxic treatments should be leached in fresh water at 80°C for 16 days.
2. Creosote treated panels covering the same treatment range as the sample panels should receive the same leaching treatment.

3. The sample panels and creosoted panels should be exposed simultaneously for six months.

4. Upon inspection at the end of this period, damage should be plotted as $L + 2T$ as a function of treatment (in lbs. retention/ft³). The rating is from 0 to a maximum of 5, depending upon extent of the area damaged, for each organism. L represents Limnoria attack and T represents Teredid attack, thus weighting Teredid attack to offset the abnormal Limnoria damage due to the size and shape of the test panels, which present great surface area per unit of volume.

5. From the graphical representation of damage as a function of treatment, the ratio of creosote treatment (L_c) in lbs./sq.ft. to sample treatment (L_s) in lbs./sq.ft. of whole creosote equivalent, giving equal protection should be computed at several impregnation levels. The plot of L_c/L_s of any toxic as a function of treatment may serve as an index of antiborer rating for a given toxic.

B. Results. A test of the above protocol was carried out. Panels impregnated with whole creosote, Claisen-alkali-extracted creosote, creosote fortified with fraction No. 2, and creosote fortified with fraction No. 3 were subjected to the testing procedure outlined above. Leaching periods other than 16 days were included in the experiment to further substantiate the choice of the 16 day period. Results of an inspection of the panels at the end of 6.3 months of exposure are as follows:

I. Whole Creosote
Exposed 8 Sept. 1952
Inspected 19 Mar. 1953

1 Very light attack L - Limnoria
2 Light attack
3 Moderate attack T - Teredo
4 Heavy attack
5 Very heavy attack

Days Leached	Approximate Treatment (Lbs./ft ³)							
	Untreated	2	5	10	15	20	25	30
	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>
0	5-2	3-1	3-1	2-1	1-0	1-0	1-0	1-0
1		1-1	4-1	2-1		1-1		
2				4-1		2-1		
4	5-3	3-3	4-2	3-1	3-1	3-1		
8				3-1		3-2		2-1
16	5-1	5-4	5-1	4-2	3-1	2-1	2-2	2-0
32	5-3			4-1	4-1	4-1		1-0
48				4-1	4-2	3-1	3-2	3-1
60	5-2			3-1	4-1	3-1	3-1	2-1
72	5-5			5-2	2-2	4-2	3-1	3-1

II. Claisen-alkali-extracted creosote
exposed 8 Sept 1952
Inspected 19 Mar 1953

Days Leached	Approximate Treatment (Lbs./ft ³)						
	<u>2</u>	<u>5</u>	<u>10</u>	<u>15</u>	<u>20</u>	<u>25</u>	<u>30</u>
	<u>L T</u>	<u>L T</u>	<u>L T</u>	<u>L T</u>	<u>L T</u>	<u>L T</u>	<u>L T</u>
0	1-0	1-1	1-1	1-0	0-0	0-0	0-0
1	5-2	3-1	1-0		3-1	1-2	
2				2-3		1-0	
4	5-2	5-1			3-1	2-0	
8	3-3	3-2	3-1	2-0	2-1	2-1	
16	3-2	5-2	3-1	3-1	2-1	1-0	
32			4-1	3-2	3-1	3-1	3-1
48		5-1	5-2	5-1	2-1	3-1	2-1
60				4-3	4-2	3-2	4-1
72				4-1	3-1	4-1	3-1

III. Creosote fortified with Fraction No. 2 so as to double content of this fraction.

Exposed 8 Sept. 1952

Inspected 19 Mar. 1953

Days Leached	Approximate Treatment (Lbs./ft ³)						
	2	5	10	15	20	25	30
	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>
0	5-3	4-2	3-2	4-1	1-1	1-1	1-1
1	4-2	3-1	3-1				
4	5-2	3-1	3-1	3-1			
16	4-2	4-2	3-1	3-1	2-0	2-0	
48				4-1	4-2	3-1	2-1
72					4-1	4-3	

IV. Creosote fortified with Fraction No. 3 so as to double content of this fraction.

Exposed 8 Sept. 1952

Inspected 19 Mar. 1953

Days Leached	Approximate Treatment (Lbs./ft ³)						
	2	5	10	15	20	25	30
	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>	<u>L</u> <u>T</u>
0	4-1	5-0	3-1	1-0	1-0	1-0	0-0
1	4-1	5-1	4-1		4-1		
4	3-2	4-2	3-1	3-1	2-1		
8	4-2	3-1			2-1	1-0	
16	5-4	5-2	5-2	3-1	3-1	3-1	
32				4-1	3-1	2-1	
48				4-1	4-1	4-2	3-1
60				4-1	3-1	4-1	
72					2-2	3-3	

Figures 3, 4, 5 and 6 illustrate the behavior of each toxic when subjected to different periods of accelerated leaching. Figure 7 illustrates

the relationship of damage to treatment with respect to unleached panels.

L_c/L_g values were computed from the curves in figure 8. These anti-borer ratings are plotted for the three samples tested (fig. 9).

On the basis of these results (fig. 7), it appears that, at all degrees of retention, the panels impregnated with Claisen alkali extract were superior to attack than standard creosoted panels prior to leaching. Subsequent to 16 days leaching their behavior compared to creosoted (fig. 9) showed the extract to be superior at low and high retentions, but inferior at intermediate levels.

Creosote fortified with fraction 2 appeared decidedly inferior to standard creosote prior to leaching but superior after leaching. On the other hand, creosote fortified with fraction 3 did not differ, on the average, from creosote either before or after leaching.

Examination of the above results indicates a considerable range of variation in numerical data and throws doubt upon the usefulness of the test as a method of evaluating the relative effectiveness of creosote fractions.

Comparison of unleached panels is difficult because of their initial high resistance to attack and it was for this reason that the leaching technique was originally proposed, in the hope that resistance might diminish uniformly. Apparently this is not so.

Further experiments are being continued before drawing final conclusions as to the value of leaching prior to field exposure tests.

Leached panels impregnated with creosote fortified with fractions 4, 5, 6 and fractionation residue were exposed on 12 December 1952 and are to be inspected at the end of six months of exposure.

Panels impregnated with base-free and acid-and-base-free creosote have been leached but have not as yet been exposed.

7. Miscellaneous Panel Exposure Tests

1. Panels impregnated with chlorinated creosote prepared by NRL were exposed 28 January 1953. An inspection on 24 March 1953 showed no evidence of Teredo or Limnoria attack on either chlorinated creosote- or whole creosote treated panels.

2. Damage to the original holiday panels after three months of exposure was as follows:

Size of Holiday	Damage to treated and untreated areas					
	Creosote		Antifouling Paint		White Deck Paint	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
1/8"	F-2	F-0	F-0	F-0	F-1	F-1
	L-0	L-1	L-0	L-0	L-1	L-1
	T-0	T-0	T-0	T-0	T-0	T-1
1/4"	F-2	F-2	F-0	F-0	F-1	F-1
	L-0	L-1	L-0	L-0	L-1	L-1
	T-0	T-0	T-0	T-0	T-1	T-1
1/2"	F-2	F-2	F-0	F-0	F-1	F-1
	L-0	L-2	L-0	L-0	L-1	L-1
	T-0	T-1	T-0	T-0	T-1	T-1

F, Fouling; L, Limnoria; T, Teredo

New panels having holidays of 1/8", 1/4", 1/2", and 1" prepared by NRL have been exposed and inspected periodically to determine the extent of damage and toxic diffusion. No results are yet available.

3. Unleached creosoted blocks serving as a long term field test for accelerated leaching tests were exposed 24 April 1951. Inspection on 22 January 1953 showed the following:

1" x 2" block (30.7 lbs./ft ³)	Fouling	Heavy
	<u>Limnoria</u>	- No attack
	<u>Teredo</u>	- No attack
2" x 4" block (26.6 lbs./ft ³)	Fouling	- Heavy
	<u>Limnoria</u>	- No attack
	<u>Teredo</u>	- No attack

4. Erdalith-treated panels were exposed 18 April 1951. Inspection on 22 January 1953 showed the following:

<u>Erdalith Retention</u> <u>(lbs./ft³)</u>	<u>Fouling</u>	<u>Limnoria</u>	<u>Teredo</u>
1.49	4	0	0
1.46	4	0	0
1.55	4	0	0
2.41	4	0	0
2.58	3	0	0
2.67	2	0	0
Untreated control	4	5	5 (destroyed)

A new untreated control panel was attached 22 January 1953.

5. A panel of Greenheart (*Ocotea rodiaei*) was exposed on 1 June 1950. Inspection on 22 January 1953 showed the following:

Fouling - Very heavy
Limnoria - Four small burrows - very light attack.
Teredo - Scattered pits and young burrows. Six large burrows.

6. Silica-containing Surinam timber panels (1" x 4" x 12") were exposed on 7 February 1951. Inspection on 22 January 1953 showed the following:

<u>Local Name</u>	<u>Fouling</u>	<u>Limnoria</u>	<u>Teredo</u>
Bongro Foengoe	Lost May 1952, no attack		
Jan Snijder	"	"	"
Anaura	"	"	"
Anaura	4	0	1
Savanna Foengoe	4	3	3
Foengoe	Lost May 1952, no attack		
Man Foengoe	4	2	2
Sopohoedoe	Destroyed by <u>Teredo</u> , June 1952		
Zwarte Riemhout	4	1	1
Witte Riemhout	4	4	3
Manbarklak	3	1	1

7. A composite panel, consisting of an untreated panel bolted to a creosoted 2" x 4" block, was exposed on 3 June 1950. On 22 January 1953 a few fragments of the untreated panel remained. The interface between the two panels was perforated by burrows, but none crossed into the creosoted wood. The creosoted block showed no attack of Limnoria or Teredo. A new untreated panel was attached to the creosoted block.

8. Publications:

The following papers have been published during the period covered by this report:

Lane, Charles E., Posner, G. S. and Greenfield, L. J.. The Distribution of Glycogen in the Shipworm, Teredo (Lyrodus) pedicellata Quatrefages. Bull. Mar. Sci. Gulf & Caribbean, 2(2):385, (1952).

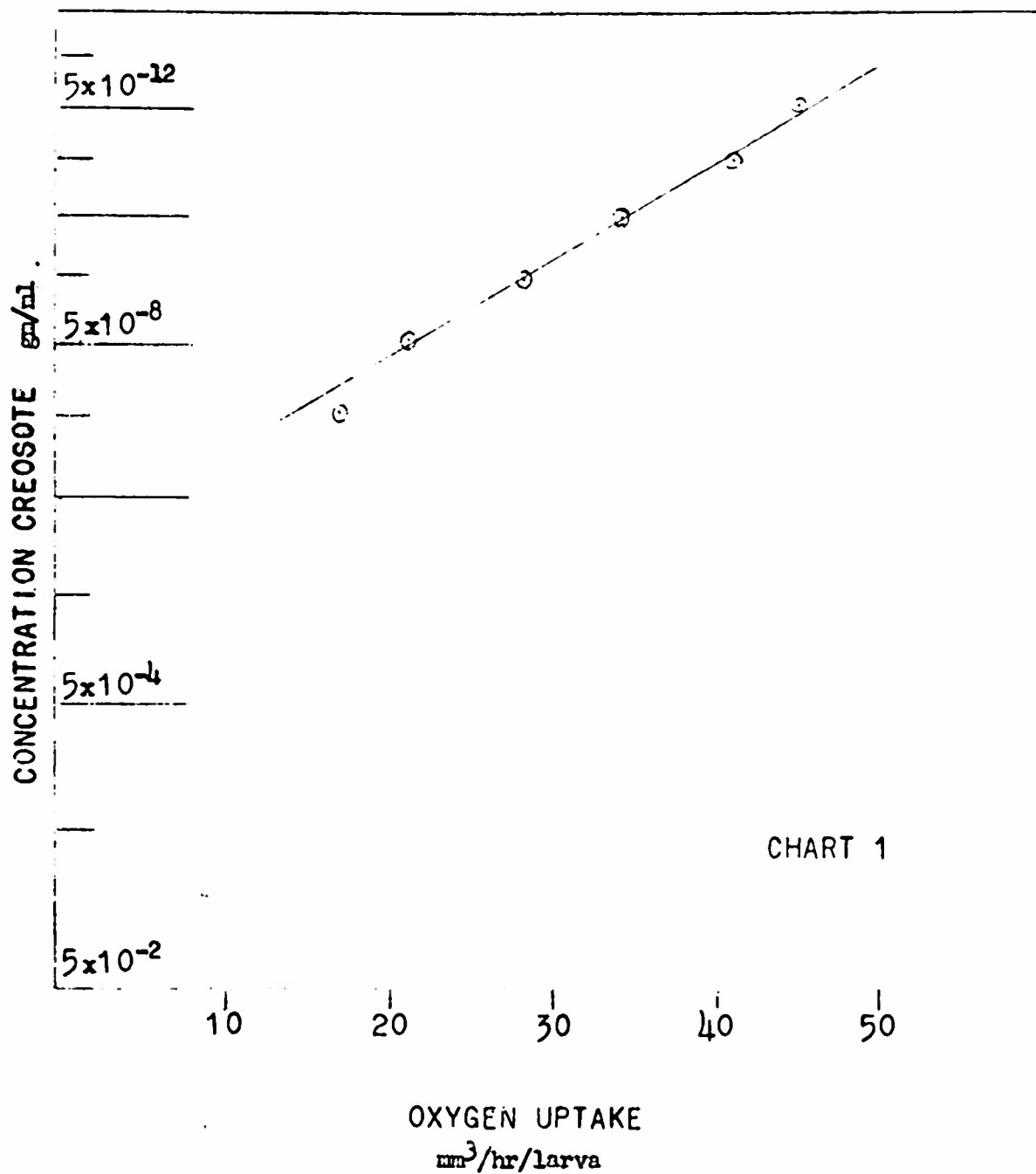
Greenfield, L. J. The Distribution of Marine Borers in the Miami Area in Relation to Ecological Conditions. Bull. Mar. Sci. Gulf & Caribbean, 2(2):448, (1952).

The following papers are either in press, or have been submitted for publication:

Lasker, R. and Lane, Charles E. Origin and Distribution of Nitrogen in Teredo, submitted to the editors of Physiological Zoology.

Greenfield, L. J. and Lane, Charles E. Cellulose Digestion in Teredo, submitted to the editors of the Journal of Biological Chemistry.

6-27-1



EXPOSURE PERIOD (months)

1

2

3

4

6

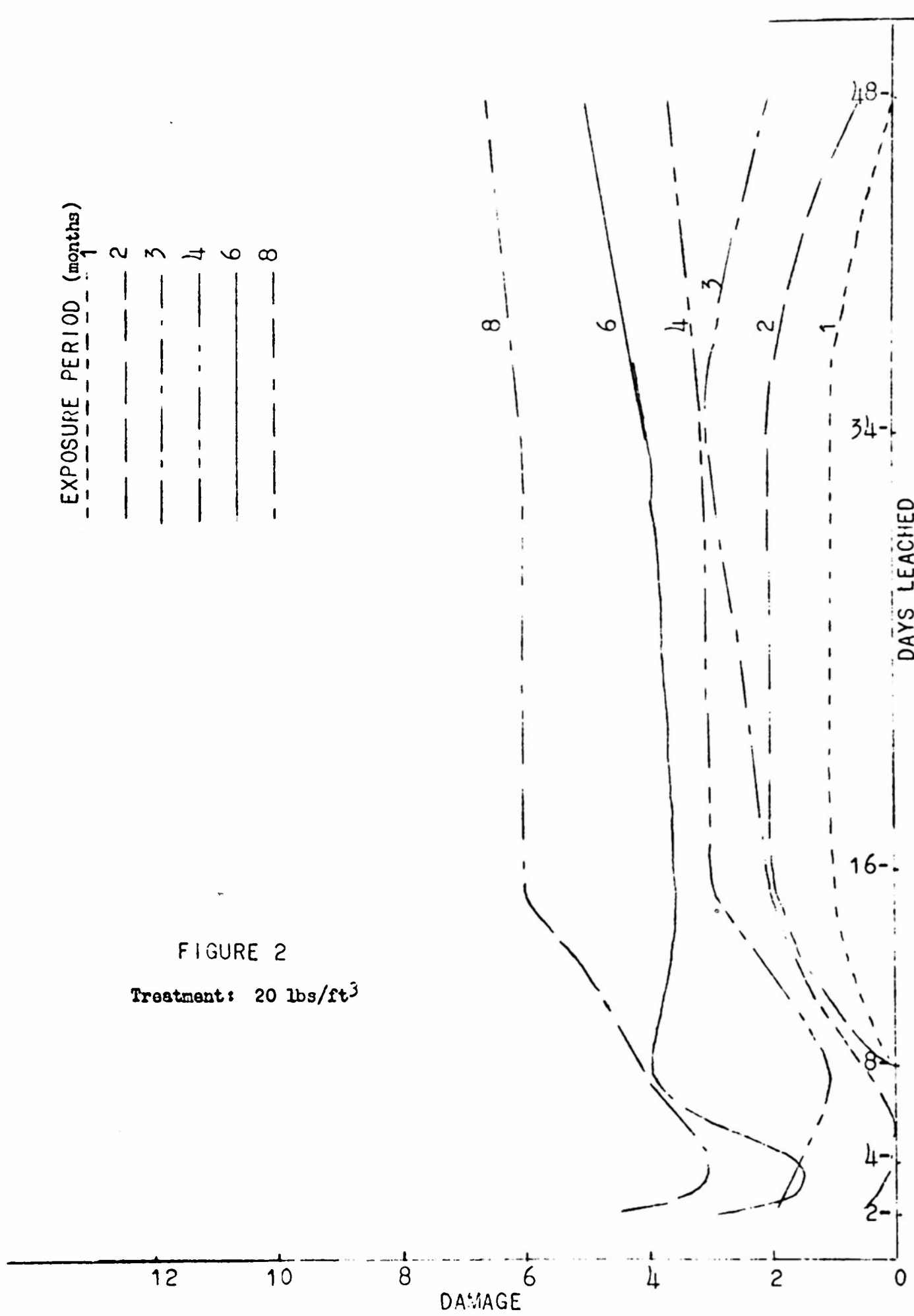
8

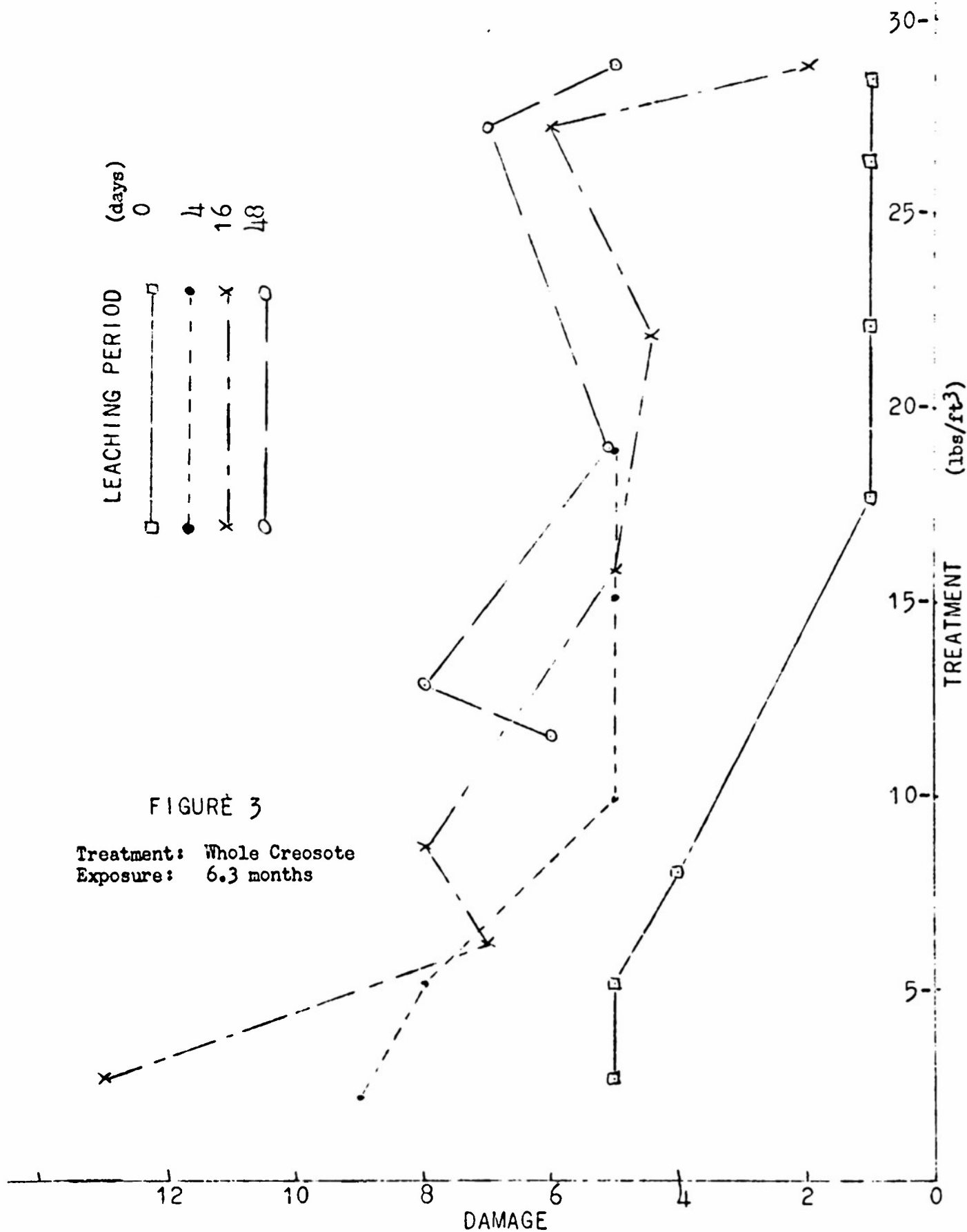
FIGURE 2

Treatment: 20 lbs/ft³

DAMAGE

DAYS LEACHED

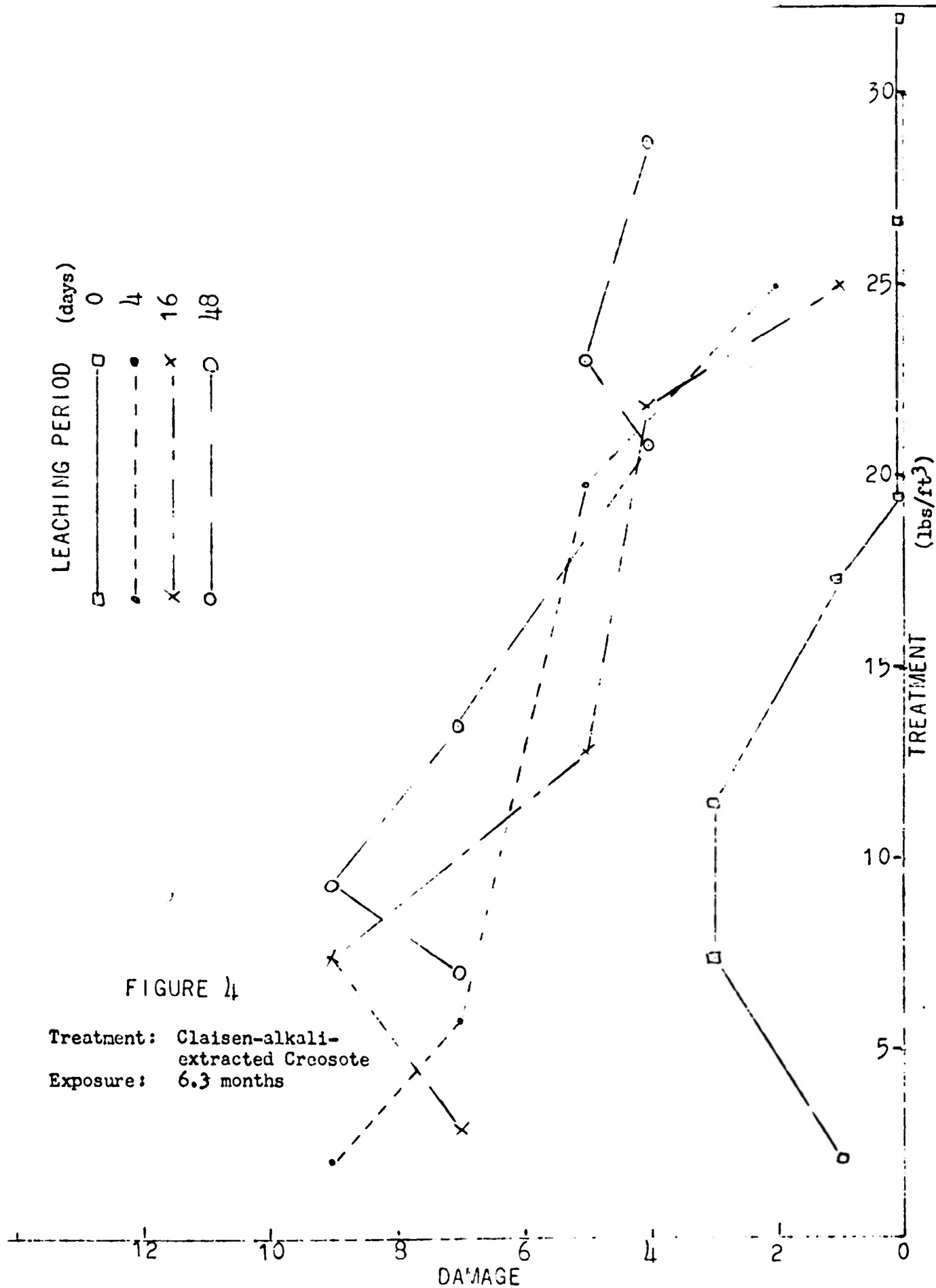




LEACHING PERIOD	(days)
□	0
●	4
x	16
○	48

FIGURE 4

Treatment: Claisen-alkali-
extracted Creosote
Exposure: 6.3 months

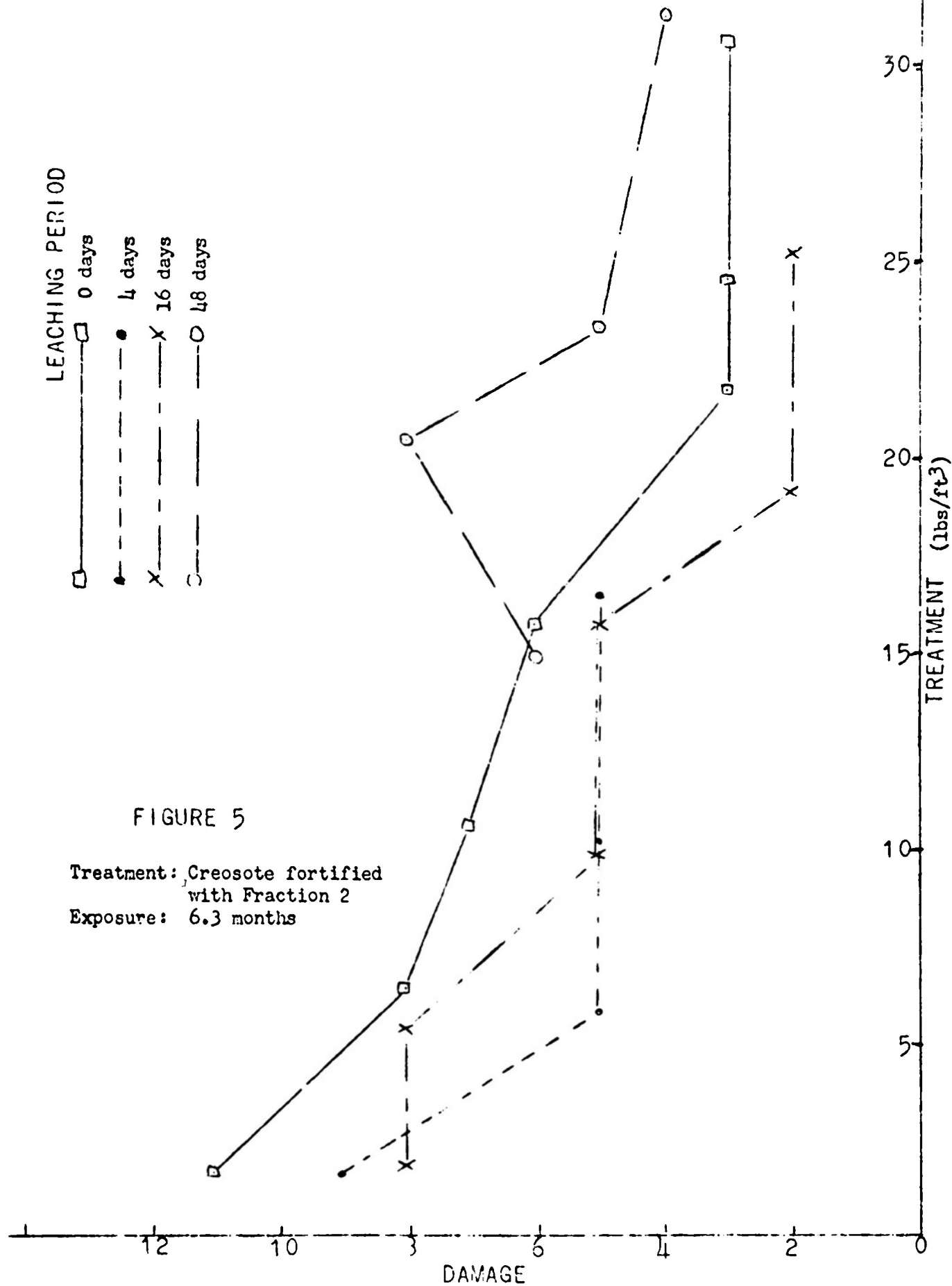


LEACHING PERIOD

- 0 days
- 4 days
- 16 days
- 48 days

FIGURE 5

Treatment: Creosote fortified
with Fraction 2
Exposure: 6.3 months



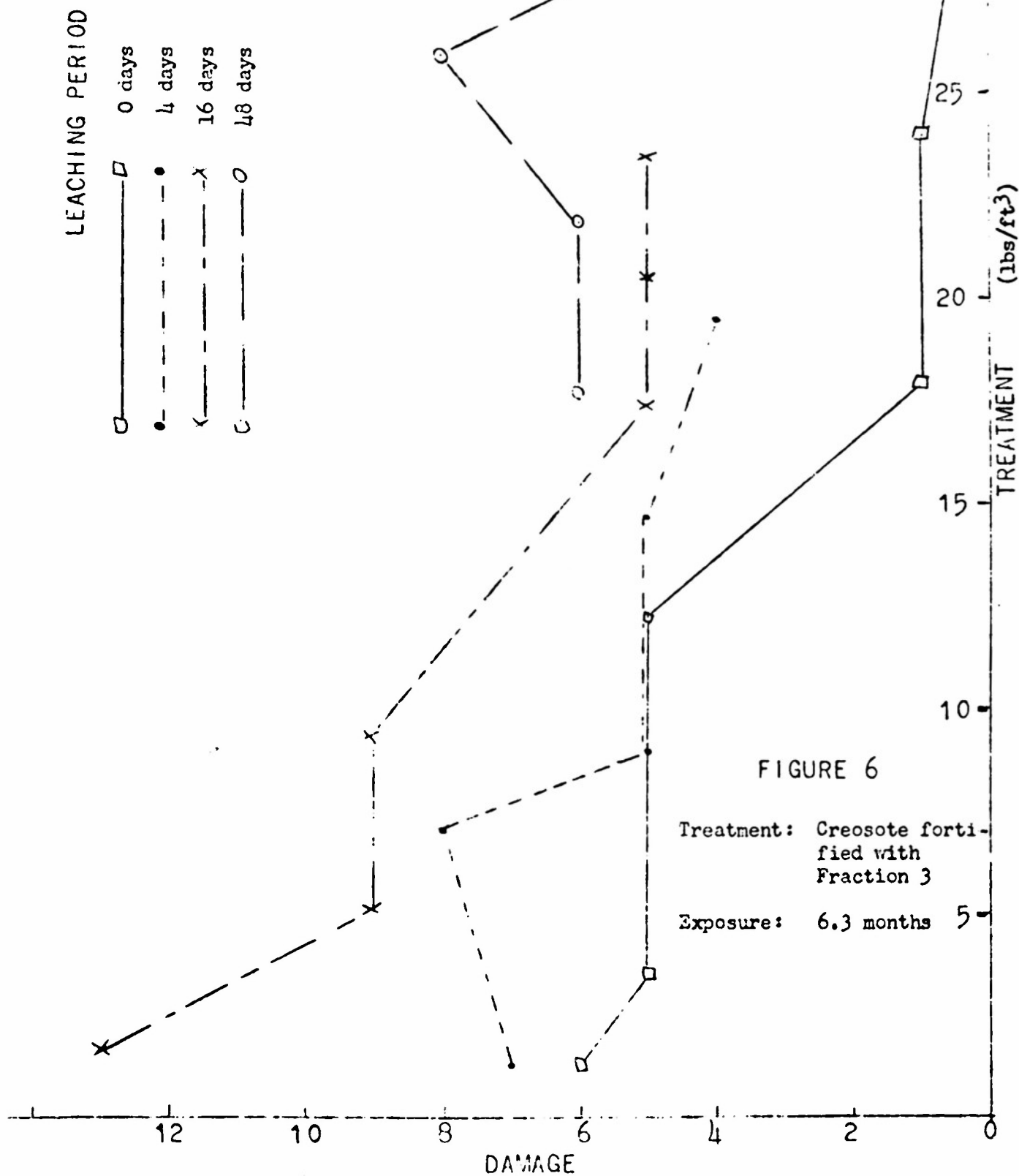


FIGURE 7

Exposure: 6.3 months
Leached: 0 days

